Signaling Pathways for the Fluid Shear Stress Induction of Cyclooxygenase-2 in Murine Osteoblats

S. Wadhwa*[1], S. Godwin[1], L. G. Raisz*[2], C. C. Pilbeam*[2]. [1]Oral Biology, University of Connecticut Health Center, Farmington, CT, USA, [2]Medicine, University of Connecticut Health Center, Farmington, CT, USA.

Orthodontic treatment relies on the predictable response of bone to mechanical loading. The rate of tooth movement can be repressed by inhibition of prostaglandin (PG) production. Fluid shear stress (FSS), hypothesized to transduce mechanical loading into cellular signals, generates PGs in osteoblasts by inducing new gene transcription of cyclooxgenase-2 (COX-2). To examine signaling pathways involved in this induction, we used immortalized murine osteoblastic MC3T3-E1 cells stably transfected with -371/+70 bp of the murine COX-2 5' flanking DNA fused to a luciferase reporter (Pluc) and primary osteoblastic cells derived from calvaria of mice transgenic for Pluc. Cells were plated on collagen-coated glass slides, grown to confluence, and subjected to 10 dynes/cm^2 of steady laminar FSS in a parallel plate flow chamber. On Northern blot analysis, COX-2 mRNA was induced by FSS within 30 minutes and peaked at 4 h. An inhibitor of new protein synthesis, puromycin (10 μg/ml), did not affect the small FSS induction of COX-2 mRNA at 1 h but inhibited the large peak induction at 4 h by 90%. COX-2 promoter activity, measured as luciferase activity normalized to total protein, correlated with COX-2 mRNA expression. Inhibitors of the protein kinase A (PKA) signaling pathway, H-89 (30 μM) and PKI (1 μM), used at doses determined to be most specific for the PKA pathway, reduced FSS stimulated COX-2 mRNA expression and luciferase activity at 4-4.5 h by 60% and 66%, respectively. In contrast, a specific inhibitor of the protein kinase C (PKC) pathway, GF109203X (1.25 μg/ml), and down regulation of the PKC pathway by 24 h of pretreatment with phorbol myristate acetate (1 μM) had no effect on the FSS induction of COX-2 mRNA and luciferase activity at 4-4.5 h. On Western analysis, FSS induced phosphorylation of ERK1/2 within 5 min. A specific inhibitor of the ERK1/2 pathway, PD98059 (40 μM), reduced FSS stimulation of COX-2 mRNA and luciferase activity at 4-4.5h by 65% and 91%, respectively. We conclude that maximal induction of COX-2 by FSS requires new protein synthesis, involves the PKA but not the PKC pathway, and depends on activation of ERK1/2. Part of this work has been published in Journal of Bone Mineral Research. 2002 Feb;17(2):266-74.